



Model 373A
Version 2.0.18

B579 20
Dye Terminator (Any Primer)
Lane 20
Signal: G:757 A:1184 T:300

INST FILE 808286
096-1369
MCGRAW-A-2F-1

Tue, Mar 5, 1986 8:51 PM
X: 0 to 6634 Y: 0 to 1600
Spacing: 10.40

Page 1 of 1
A3-f

[illegible]

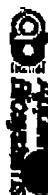
AAAGGAGGATGACCTGACCCCTTGGCGAGTCCCGAGGTGACCATTTGCCGGAAGATTCGCCATACTGACGCGGAGGCGAATTCGCCCTAAATCCTGTGCACCATAAAGG

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CAGGCACACACA³⁴⁰GAGCCGGCTGA³⁵⁰ATGAAAGCTTCGAGGCGATCCGCGTGGCGCGGCCGGA³⁶⁰AAACCCCGCGCGATGAGGTCCAGCC³⁷⁰CTGCTGAAG³⁸⁰CCCCCAAC³⁹⁰GTGAGC

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Model 373A
Version 2.0.1S

B5/9 16
Dye Terminator{AnyPrimer}
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Signal: G:1186 A:1244 T:5

INST FILE 808296
086-1364
MOGRAW--A-2/R--

Tue, Mar 5, 1996 8:51 PM
X: 0 to 6640 Y: 0 to 1600
Spacing: 10:38

Page 1 of 1

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Model 373A
Version 2.0.1S

55/9 18
Dye Terminator (Any Primer)
Lane 18
Signal: G:1128 A:1741 T:5

INST FILE 808298
098-1367
MCGRAW--DWMF-1

Tue, Mar 5, 1986 8:51 PM
X: 0 to 6615 Y: 0 to 1600
Spacing: 10.43

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[illegible]



Model 373A
Version 2.0.1S

B5/914
Dye Terminator (Any Primer)
Lane 14
Signal: G:191 A:1167 T:5

INST FILE 808296
098-1362
MCGRAW--DWM/R-1

Tue, Mar 5, 1996 8:51 PM
X: 0 to 6650 Y: 0 to 1600
Spacing: 10.47

Page 1

DM-R

CAC AAGAA GGGCT CCGGT TGGGAGT TGGCC ATGCGCGCGCAT CTGACAGT AAGCGACT GAGCTGGGAGCGCCCTCAAGGAGCGA ACCGACCGACGAGGCT CTGCGCGG

1800G AAGCGAAGGGCTCATTCAGGGGCTGTGATGATGAGATGTGAAGGAGNCTGALGOTACACAATCAGAGCTTATTGGAGTAGCTAAAGGGGCTCACCAATACCTAGCCA
 190
 200
 210

228 C A G C C G G T G G T G T G A A G A A G C C C G C T T C G G A G T A G C G T T C T G C C C G A T T A T G T C C A G A G G A G C T T T A G A G G A G C T T G C C C T C G C G T G A G A C T A C C G G A A C T T T C G G C C C

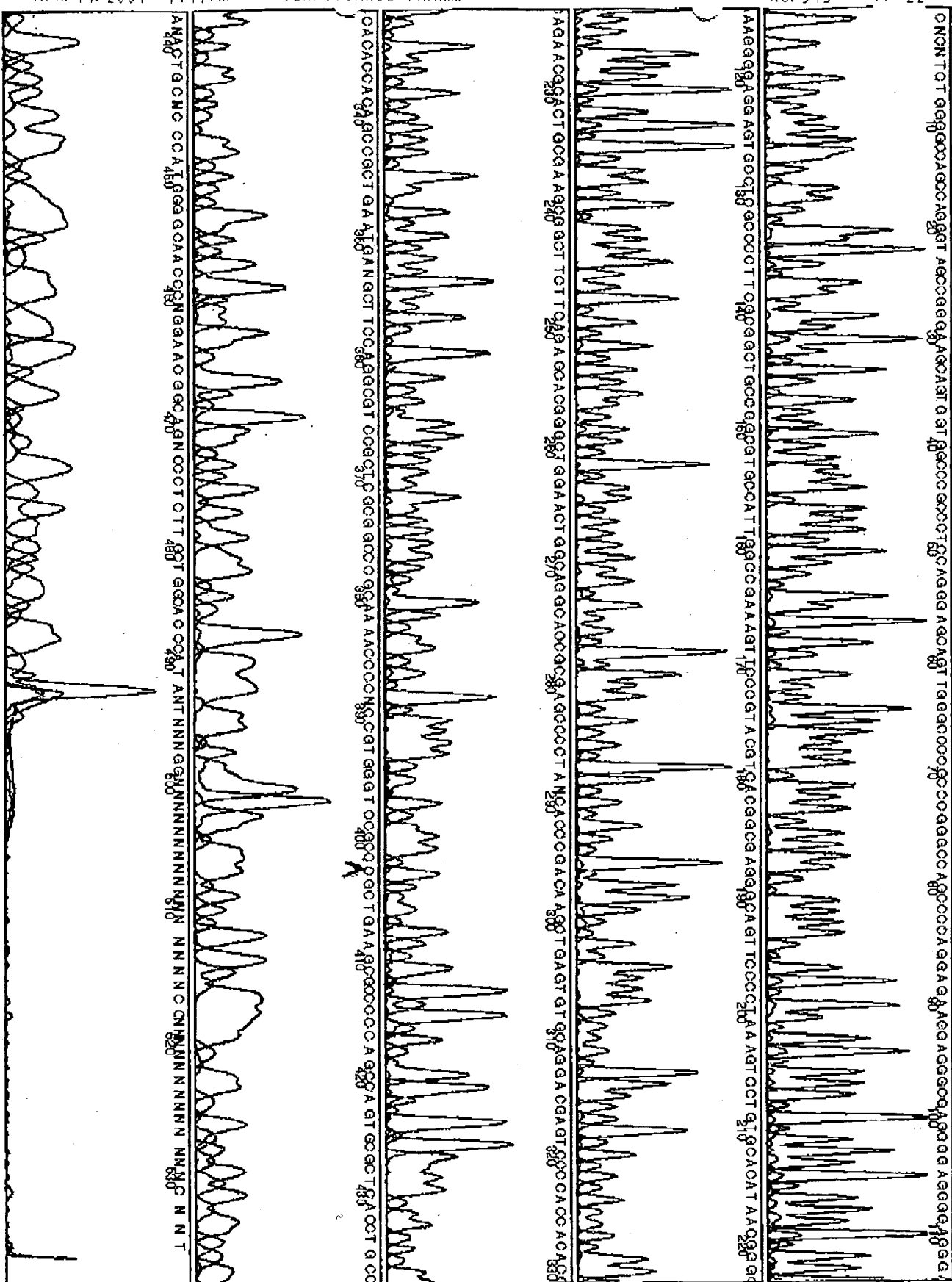
A T A G G A C G C C A G G C A G G C G C C A A A G G G C G A A G G C A G C A G C T C T C C C C T T T C C C C T C C C C T T G C C C C C C C C C C C T T C T C C T C T G A G C T A G C C G N G A G C N A G C C G A A C T G C
 380 390 400 410 420

[illegible]

NNNNNNNNNNCC
830

Model 373A
Version 2.0.1SBioss UB
Dye Terminator/Any Primer
Lane 8
Signal: G:406 A:399 T:97 C:121INST FILE 808296
096-2859
MCGRAW/HAG/F1Thu, May 9, 1996 4:57 PM
X: 0 to 6472 Y: 0 to 1600
Spacing: 10.26

Page 1





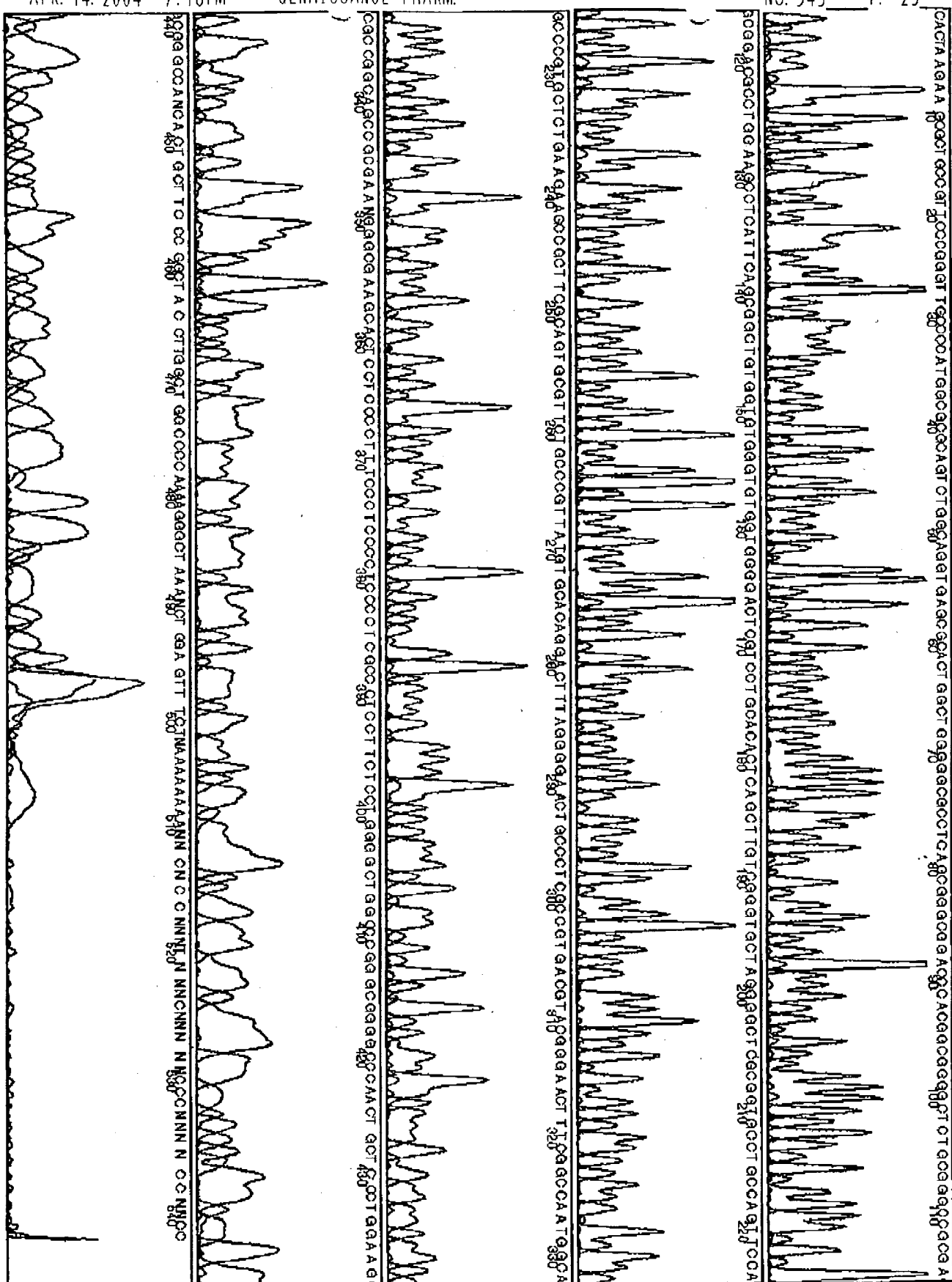
Model 373A
Version 2.0.13

H6335 09
 DyTerminator(AmyPrimer)
 Latre 9
 Signal: G:315 A:191 T:85 C:85

INST FILE 808296
098-2860
MCGRAW/HAS/R1

Thurs, May 9, 1996 4:57 PM
X: 0 to 6452 Y: 0 to 1600
Spacing: 10.31

Page 1



PCR (B2AR)

1/26/96

* Made new set of primers for B2AR 5' flanking region. ~~Best~~
Product should include the CRE + short ORF. Primers
were chosen = MacVector

Blood samples are being obtained from pts in the UC cell
clinic by Melanie Meyers.

DNA samples prepared by Liz Donnelly using ~~Amplify~~ ^{Amplify} +/m
Diana kits. Samples are numbered as received (A1, A2, etc.)

- Set up PCR rxn using new primers + made up 96ul master mix.

10ul buffer II

6ul 25mM MgCl₂

0.8ul 25mM dNTP

0.5ul 100uM forward primer (B2AR-F1)

0.5ul 100uM reverse primer (B2AR-R1)

77.7ul dH₂O

0.5ul amplitag

- digest 24ul of master mix into 2 PCR tubes

- add 1ul template DNA

- overlay = 1 drop mineral oil

- perform PCR in thermal cycler = denat gradient made on

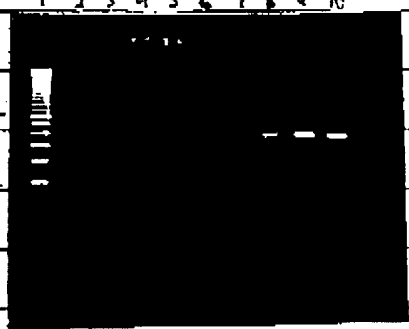
94°C x 2 min

94°C x 30 sec / 64°, 63°, 60°, or 58°C x 30 sec / 72°C x 30 sec => 35 cycles

72°C x 7 min

- run 10ul of PCR rxn on TBE minigel

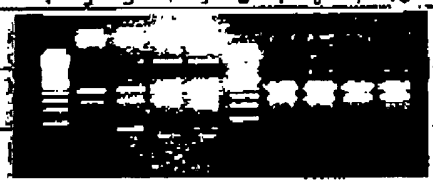
1/26/96 (cont)

	1	2	3	4	5	6	7	8	9	10
see GAK RIA										
reaction	600									
reaction time (1.20.46)	100									
	1 - 100 bp ladder					6 - blank				
	2 - A1 (Amplify) 64°C					7 - A1 (Amplify) 64°C				
	3 - " 62°C					8 - " 62°C				
	4 - " 60°C					9 - " 60°C				
	5 - " 58°C					10 - " 58°C				

No product seen = DNA isolated by Amplify kit. Nice band seen = DNA from Qiagen kit but expected size should be 538 bp. The band present appears to be <400 bp.

Repeat PCR with following modification:

- add 73.7 ul dH₂O
- digest 23 ul of master mix into PCR tubes
- add 2 ul template DNA
- change cycling temp for Amplify amplase to 56°, 54°, 53° + 50° C; change cycling temp for Qiagen amplase to 58°, 56°, 54°, 52° C.

	1	2	3	4	5	6	7	8	9	10
										
	1 - 100 bp ladder					6 - 100 bp ladder				
	2 - A1 (Amplify) 58°C					7 - A1 (Amplify) 58°C				
	3 - " 54°C					8 - " 56°C				
	4 - " 52°C					9 - " 54°C				
	5 - " 50°C					10 - " 52°C				

Now have bands in all samples. However, apparent band present in both samples sets appears to be <500 bp, still smaller than expected.

3/1/96

B2AR PCR (5 μ l)Set up master mix for four (4) 25 μ l PCR rxns:

2 μ l template DNA (DNA)
 1.5 μ l forward primer
 1.5 μ l reverse primer
 20 μ l 5X buffer (buffer A from Phastagene PCR optimization kit)
 10 μ l dUTP_s (2.5mM) (from optimization kit)
 65 μ l dH₂O
 0.8 μ l tag
 100 μ l total

- aliquot 25 μ l of master mix into 4 PCR rxn tubes- overlay \approx 1 drop of mineral oil

- PCR cycle 98°C 2 min

98°C 30 sec

56°C 54°C 52°C 50°C 30 sec

72°C 30 sec

72°C 7 min

30 cycles

- remove 10 μ l aliquot & run on minigel

#1 - 100 bp ladder

#2 - 56°C

#3 - 54°C

#4 - 52°C

#5 - 50°C

